Evolution of morphogenesis can drive the emergence of stem to non-stem cell differentiation

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Abstract

Multicellularity is characterised by the irreversible differentiation of stem cells to non-stem cells. This study explores the re-occurrence of this irreversible differentiation in the context of multicellular evolution. Current theoretical research assumes that irreversible differentiation occurs when a cell specialises heavily in a somatic tasks. We question this assumption by investigating the role of morphological evolution on irreversible differentiation using a multi-scale computational model. Our findings reveal that the emergence of stem to non-stem cell differentiation (stem-cell-system) that is closely associated with the evolution of morphology. Moreover, we demonstrate a bidirectional relationship, as organisms with stem-cell-systems have much more reproducible morphologies. By elucidating the link between morphological evolution and the presence of stem-cell-systems, this research offers valuable insights into the fundamental mechanisms driving the evolution of multicellular complexity.

Introduction

Cell differentiation, a fundamental characteristic of multicellular organisms, involves the transformation of a cell's phenotype, resulting in the transition from one cell type to another. The majority of differentiation events in multicellular organisms occur through the irreversible differentiation of stem cells to non-stem cells. Stem-cells are capable of maintaining their population via self-renewal, whereas the non-stem, differentiated cells become stuck in their cell-type. These "stem-cell-systems" occur in all contexts of complex multicellularity, including the development of organisms, the organs of adult vertebrates, or asexual reproduction in many animals (Wolpert et al., 2015). The prevalence of stem-cell-systems raises a compelling question in evolutionary biology: what underlies their widespread occurrence? Existing research assumes that irreversible differentiation is a consequence of cells specialising heavily in a single task (Willensdorfer, 2009; Rueffler et al., 2012; Goldsby et al., 2012), such as oxygen transport or conducting electrical signals. Through high levels of specialisation, the cell loses the ability to differentiate. However, irreversible differentiation can occur without this specialisation. One example is in the developing embryos of animals, where stem cells create the morphology of an organism by dividing and then irreversibly differentiating. In fact, there are many stem-cell-systems that are purposed towards morphogenesis (Wolpert et al., 2015). Since morphogenesis is fundamental to multicellularity, we investigated whether there is a relationship between morphology evolution and stem-cell-systems, using a multi-scale computational model of multicellular developmental evolution.

Model

To simulate morphological evolution, we modelled a population of 60 primitive multicellular organisms that evolves over discrete generations. We selected for organism deformation from a circle as well as organism curvature. Each organism begins as a ball of 64 cells developing on a two-dimensional grid, following the Cellular Potts Model (CPM) formalism (Graner and Glazier, 1992; Hogeweg, 2000). In the CPM, biological cells are represented as a collection of cellular automaton pixels. Cell division occurs when a cell reaches a size of 100 pixels. The central aspect of the CPM is pixel copy attempts that occur at the boundary between cells, or between cells and the external medium. The acceptance of a pixel copy attempt is determined by its effect on the system's energy, denoted as H, given by

$$H = \lambda \sum_i (v_i - V_i)^2 + \lambda_2 \sum_i (l_i - L_i)^2 + \sum_{\langle x,x' \rangle} J_{x,x'} + \sum_{\langle x,m \rangle} J_{x,m}$$

where V_i and L_i are size and length constraints imposed on cell i with current size v_i and length l_i. $J_{x,x'}$ computes the surface energy that arises through cell-cell contacts, where x and x' are neighbouring pixels that belong to different cells. Similarly, $J_{x,m}$ computes the surface energy between cells and the medium, where x and m are neighbouring cell and medium pixels. If a pixel copy attempt were to increase H, it is accepted with probability $e^{-\frac{\Delta H}{T}}$, where ΔH represents the change in energy and T is the temperature parameter. If ΔH is negative, the pixel copy attempt is invariably accepted.

The values of $J_{x,x'}$ and $J_{x,m}$ are determined by adhesion proteins, and L_i by length proteins. Each cell expresses its own proteins. The expression pattern of a cell is referred to as the *cell-state*. Interactions between cells with different states allows for the unique mechanical interactions that generate organism morphology. Protein expression is determined by a gene regulatory network (GRN), which is the same for all cells within the organism. The GRN functions as a continuous extension of a boolean network. The GRN is essentially the genome of the organism, and is mutated between organism generations. The concentration (x_k) of protein k is numerically integrated by the following differential equation:

$$\dot{x}_k = \frac{1}{1 + e^{-y}} - d x_k$$

where d is the protein decay rate and y is the summed regulatory effect of nine regulatory proteins on protein k. Three of the regulatory proteins can diffuse between cells (i.e., morphogens), allowing the grid location relative to surrounding cells to change the cell-state. Changes in cell-state over developmental time allows for stem-cell-systems to emerge through irreversible cell-state changes.

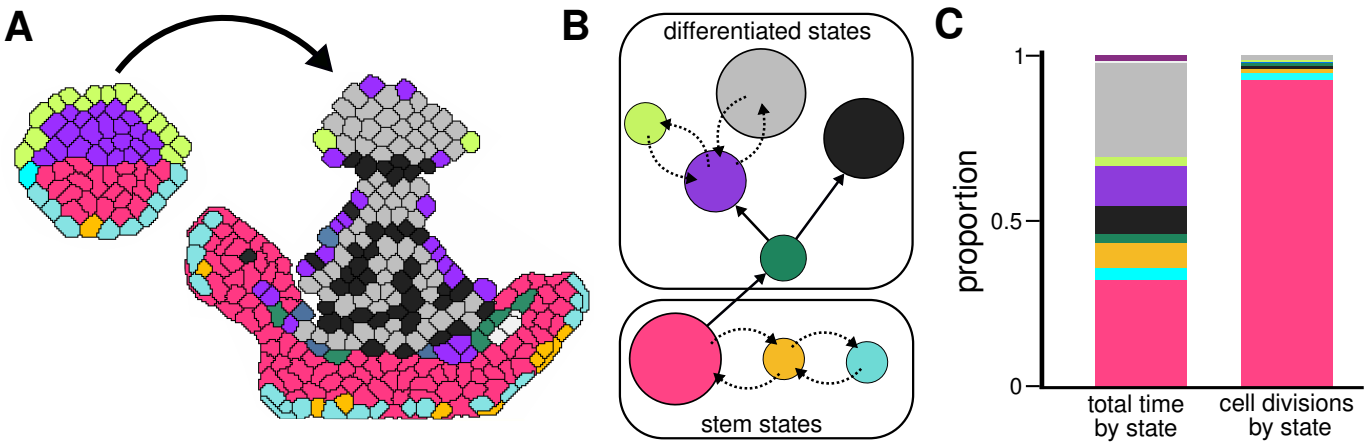

Figure 1: Evolution of a stem-cell-system. **A** The initial and final stages of an evolved organisms development on the CPM grid. Each enclosed region of a single colour is a cell; the colour depicts the state of the cell. **B** A simplified state space of the same evolved organism, illustrating irreversible differentiation from stem-cell-states to differentiated states. Filled arrows indicate irreversible differentiation; dotted arrows indicate reversible differentiation. The size of the nodes correlates with the number of cells in each respective state. **C** The stem-cells of evolved organisms are self-renewing. Despite occupying a smaller proportion of total developmental time, the vast majority of cell divisions occur within the stem-cell-states.

Results & Discussion

We ran simulations of our model for at least $3,000$ generations. Inclusion in our analysis was based on reaching a fitness threshold, which occurred in 68.9% of simulations, ($n = 116$). Once a sufficiently complex morphology evolved, subsequent evolutionary changes resulted in minimal morphological alterations, indicating the attainment of a stable morphology through evolution. We selected the genome with the highest organism fitness at the end of each simulation (referred to as evolved organisms) for analyses, each of which displayed a unique morphology. An example of an evolved organism is depicted in Figure 1A.

In our exploration for the prevalence of stem-cell-systems in evolved organisms, we probed for the existence of two prerequisites: irreversible cell differentiation and stem-cell self-renewal. To test for irreversible cell differentiation, we recorded how cells change state during the development of each evolved organism (i.e., the state space, shown in Figure 1B). Stem-cell self-renewal was tested by determining whether the stem population was stable over time (Figure 1C). This analysis showed that stem-cell-systems were present in 26.8% of evolved organisms, compared to less than 0.1% of organisms with randomly generated genomes ($n = 1819$), which functions as a control. This led us to conclude that the evolution of stem-cell-systems is facilitated by morphological evolution.

We next questioned whether stem-cell-systems had a tangible effect on morphology, instead of emerging simply as a side-effect of evolving complexity. We noticed that organisms with stem-cell-systems were able to reproduce their morphology when their development is replayed more consistently than evolved organisms without stem-cell-systems (morphology is highly variable due to the probabilistic nature of the CPM). The ability to reproduce morphology is a fundamental prerequisite for biological viability. To analyse reproducibility rigorously, we replayed the development of each evolved organism sixty times and measured the reproducibility of each morphology via a grid overlap measurement. We found a difference of 62.7% in organisms with stem-cell-systems to 33.2% in those without ($p < 0.001$), indicating a strong correlation between stem-cell-systems and reproducibility.

Intriguingly, the state space used to test for irreversible differentiation serves as a "homonculus" representation of the organism morphology (Figure 1A,B), but only in the context of organisms with stem-cell-systems. In the simplest sense, stem-cells grow in one direction, and differentiate in the opposing direction. Even when the state space is more complex, it still approximately depicts the organism morphology. We thought that this mirroring effect of the state space on the organism morphology may improve morphological reproducibility, because the state space remains constant each time development is replayed. As the state space remains constant, so does the morphology. Given that the state space of stem-cell-systems often exhibits directionality (from stem to non-stem), we hypothesised that selecting for organisms that demonstrate growth in a specific direction could further elucidate the connection between stem-cell-systems, morphology, and reproducibility. To test this, we conducted a new set of evolutionary simulations, incorporating an additional selection pressure for organisms that exhibit a directional shift in their center of mass over time. Notably, organisms with stem-cell-systems evolved in 92% of these simulations, compared to 20% of simulations where the shifting of mass was the sole selection pressure.

In summary, our research elucidates a novel relationship between stem-cell-systems, morphological evolution and morphological reproducibility.

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